

APPENDIX A CLAIMS
Serial No.: 10/583,179
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LISTING OF CLAIMS:

1. (Original) A protein separation device comprising a chaperone protein immobilised on a substrate.
2. (Original) The protein separation device as claimed in claim 1, in which the chaperone protein is selected from the group consisting of Hsp100, Hsp90, Hsp70, Hsp60 and small Hsps.
3. (Original) The protein separation device as claimed in claim 2, in which the Hsp60 chaperone is a group one chaperone.
4. (Original) The protein separation device as claimed in claim 3, in which the group one chaperone is GroEL.
5. (Original) The protein separation device as claimed in claim 4, in which GroEL is in a single ring configuration.
6. (Currently amended) The protein separation device as claimed in claim 4, in which GroEL comprises a back-to-back double ring configuration, ~~wherein one ring is heptameric and the other ring is dimeric, trimeric, tetrameric, pentameric or hexameric.~~
7. (Previously presented) The protein separation device as claimed in claim 4, in which GroEL comprises an heptameric ring of wild-type GroEL and a ring of another chaperonin protein.
8. (Original) The protein separation device as claimed in claim 7, in which the other chaperonin protein is rubisco subunit binding protein or CCT.
9. (Previously presented) The protein separation device as claimed in claim 4, in which GroEL comprises a double ring assembly wherein one or both rings comprise one or more subunits from other chaperonins.

10. (Original) The protein separation device as claimed in claim 9, in which each ring contains one or more of the α , β , γ , δ , ϵ , ζ or θ subunits of CCT.

11. (Original) The protein separation device as claimed in claim 9, in which each ring contains one or more subunits of rubisco subunit binding protein.

12. (Previously presented) The protein separation device as claimed in claim 9, in which each ring is a heteromeric heptamer.

13. (Previously presented) The protein separation device as claimed in claim 4, in which GroEL is in operative association with a co-factor.

14. (Original) The protein separation device as claimed in claim 13, in which the co-factor is GroES.

15. (Previously presented) The protein separation device as claimed in claim 1, in which the chaperone is obtainable from a microbial source selected from bacteria and archaea.

16. (Original) The protein separation device as claimed in claim 15, in which the microbial source is selected from the group consisting of *Escherichia spp.*, *Thermus spp.*

Streptococcus spp., *Staphylococcus spp.*, *Bacillus spp.*, *Leptospira spp.*, *Spirillum spp.*, *Lactobacillus spp.*, *Mycoplasma spp.*, *Pseudomonas spp.*, *Streptomyces spp.*, *Corynebacterium spp.*, *Bacteroides spp.* and *Clostridium spp.*

17. (Original) The protein separation device as claimed in claim 15, in which the *Escherichia spp.* microbial source is *Escherichia coli*.

18. (Original) The protein separation device as claimed in claim 15, in which the *Thermus spp.* microbial source is *Thermus thermophilus*.

19. (Original) The protein separation device as claimed in claim 15, in which the *Clostridium spp.* microbial source is *Clostridium difficile*.

20. (Previously presented) The protein separation device as claimed in claim 1, in which the substrate is a solid support of the array or bead type.

21. (Original) The protein separation device as claimed in claim 20, in which the substrate is manufactured from a plastics material.

22. (Previously presented) The protein separation device as claimed in claim 20, in which the support of the array type is provided with a surface for immobilisation of a protein of the chaperone type thereon.

23. (Original) The protein separation device as claimed in claim 22, in which the surface is comprised of moieties selected from the group consisting of nitriloacetic acid, avidin, streptavidin, carboxylates, quaternary amines, silicates, carbonyl diimidazoles and epoxides.

24. (Original) The protein separation device as claimed in claim 23, in which the surface is provided with an hydrophobic barrier coating.

25. (Previously presented) The protein separation device as claimed in claim 1, in which the biological sample is selected from the group consisting of cerebrospinal fluid, urine, nipple aspirant, other biological fluids and extracts.

26. (Previously presented) The protein separation device as claimed in claim 1, in which the biological sample is denatured.

27. – 31. (canceled)

32. (Previously presented) The protein separation device according to claim 1, said protein separation device comprising GroEL immobilised on the substrate in an optimised orientation to bind a target protein and to provide minimal steric hindrance between GroEL and the substrate.

33. (Previously presented) The protein separation device as claimed in claim 32, in which GroEL is engineered by site-directed mutagenesis to substitute Aspartate⁴⁹⁰ with a cysteine.

34. (Previously presented) The protein separation device as claimed in claim 33, in which the substituted cysteine is reacted with biotin to form a biotin-cysteine conjugate.

35. (Previously presented) The protein separation device as claimed in claim 34, in which the biotin-cysteine conjugate is reactable with an avidin or streptavidin moiety located on a surface of a substrate.

36. (Previously presented) The protein separation device according to claim 1, said protein separation device comprising GroEL immobilised on the substrate, wherein the specificity of GroEL is directed to a particular protein.

37. (Previously presented) The protein separation device as claimed in claim 36, in which GroEL is engineered by site-directed mutagenesis to have the substitutions, leucine 200 to arginine; serine 201 to glycine, and proline 202 to aspartate.

38. (Previously presented) The protein separation device as claimed in claim 37, in which the substitutions introduce an integrin binding motif into a protein binding domain of GroEL.

39. (Previously presented) The protein separation device according to claim 1, said protein separation device comprising GroEL immobilised on the substrate wherein the specificity of GroEL is changed to a protein specificity of another chaperone protein.

40. (Previously presented) The protein separation device as claimed in claim 39, in which GroEL is engineered by site-directed mutagenesis to have the substitutions, Tyrosine 199 to Isoleucine; Tyrosine 204 to Isoleucine; Leucine 234 to Isoleucine; Leucine 237 to Isoleucine; Leucine 259 to Phenylalanine; Valine 263 to Leucine and Valine 264 to Phenylalanine.

41. (Previously presented) The protein separation device as claimed in claim 39, in which the substitutions are made in the apical protein-binding domain of GroEL.

42. (Previously presented) The protein separation device as claimed in claim 40, in which the substitutions replace the substrate binding specificity of GroEL, a group I chaperone, with that of thermosome, a group II chaperone.

43. (Previously presented) The protein separation device according to claim 1, said protein separation device comprising GroEL arranged to have an optimised orientation to bind a target protein and minimal steric hindrance between GroEL and the substrate when GroEL is immobilised on the substrate and its specificity for a target protein is directed to a particular target protein.

44. (Previously presented) The protein separation device according to claim 1, said protein separation device comprising GroEL in an optimised orientation to bind a target protein and to provide minimal steric hindrance between GroEL and the substrate wherein the specificity of GroEL for a target protein is changed to a specificity of another chaperone protein.

45. (Currently Amended) A method of isolating at least one protein from a biological sample using the protein separation device of claim 1 comprising ~~the steps of:~~

- a) denaturing a biological sample containing at least one protein;
- b) applying the biological sample containing at the least one protein to a-the protein separation device according to claim 1, said protein separation device comprising a chaperone protein immobilised on a substrate.
- c) isolating the at least one protein from the biological fluid on the chaperone protein;
- d) removing the biological sample from the chaperone protein immobilised on the substrate, and
- e) obtaining the at least one protein from the chaperone protein.

46. (Currently Amended) A method of identifying a biological marker in a biological sample using the protein separation device of claim 1, comprising ~~the steps of:~~

- a) applying the biological sample containing the biological marker to a-the protein separation device according to claim 1, said protein separation device comprising a chaperone protein immobilised on a substrate;

- b) isolating the biological marker from the biological fluid on the chaperone protein;
- c) removing the biological sample from the chaperone protein, and
- d) obtaining the at least one protein from the chaperone protein immobilised on the substrate.

47. (Currently amended) A method of diagnosis using the protein separation device of claim 1 comprising the steps of:

- a) applying a biological sample from a first subject to a the protein separation device according to claim 1, said protein separation device comprising a chaperone protein immobilised on a substrate;
- b) isolating a protein from the biological fluid on the chaperone protein;
- c) removing the biological sample from the chaperone protein;
- d) obtaining the at least one protein from the chaperone protein, and
- e) comparing the concentration of the at least one protein from the first subject with a reference concentration obtained from a second subject.

48. (canceled)

49. (canceled)